expressing the zinc finger-HSV fusion under the control of a constitutive promoter. No activation of CAT gene expression is observed.

Page 42, delete the whole paragraph starting at line 7, and replace it with the following new paragraph:

However, when the same experiment is conducted in the presence of Hae III methylase, CAT expression is observed as a result of the methylation of (SEQ ID NO.: 56) GCGGCCGCG to form (SEQ ID NO.: 58) GCGGMCGCG, and consequent binding of the zinc finger transcription factor to its recognition sequence.

IN THE CLAIMS:

Kindly enter the following amended claim.

15. (Twice Amended) The method according to claim 10, wherein the linker is the sequence set forth in SEQ ID NO.: 41 or the sequence set forth in SEQ ID NO.:3.

REMARKS

Applicants submit herewith a substitute Sequence Listing in compliance with 37 C.F.R. §§ 1.821-1.825. This Sequence Listing is submitted in lieu of the Sequence Listing filed April 12, 2002. Both a paper version of the Sequence Listing and a computer readable format containing the same information as the paper version of the Sequence Listing are enclosed. The substitute Sequence Listing does not include new matter. Entry is respectfully requested.

An early and favorable Action on the merits is respectfully requested. Notification to that effect is earnestly solicited.

Should questions regarding patentability arise, the Examiner is invited to telephone the undersigned to discuss the same.

Respectfully submitted,

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Enclosure: CRF and Paper Copy of Sequence Listing

APPENDIX
MARK UP VERSION SHOWING CHANGES MADE

IN THE SPECIFICATION:

Paragraphs in the specification have been amended as indicated below.

Page 8, paragraph beginning at line 20 is amended as follows:

Preferably, the linker is (SEQ ID NO.: 41) T-G-E-K or (SEQ ID NO.: 3) T-G-E-K-P.

Page 10, paragraph beginning at line 14 is amended as follows:

The consensuses are derived from the consensus provided by Krizek *et al.*, (1991) J. Am. Chem. Soc. 113:4518-4523 and from Jacobs, (1993) PhD thesis, University of Cambridge, UK. In both cases, the linker sequences described above for joining two zinc finger motifs together, namely (SEQ ID NO.: 41) TGEK or (SEQ ID NO.: 3) TGEKP can be formed on the ends of the consensus. Thus, a P may be removed where necessary, or, in the case of the consensus terminating (SEQ ID NO.: 42) T G, E K (P) can be added.

Page 13, paragraph beginning at line 23 is amended as follows:

Figure 1a is an alignment of the amino acid sequence of the three fingers (SEQ ID NOS.: 59-61, respectively in order of appearance) from Zif268 used in a phage display library. Randomised residue positions in the α-helix of finger 2 are marked 'X' and are numbered above the alignment relative to the first helical residue (position +1). Residues which form the hydrophobic core are circled; zinc ligands are written as white letters on a black circle background; and positions comprising the secondary structure elements of a zinc finger are marked below the sequence.

Page 14, paragraph beginning at line 6 is amended as follows:

Figure 1c shows a phage ELISA binding assay showing discrimination of pyrimidines by representative phage-selected zinc fingers. The matrix shows three different zinc finger phage clones (x, y and z) reacted with four different DNA binding sites present at a concentration of 3nM. Binding is represented by vertical bars which indicate the OD obtained by ELISA (Choo and Klug, (1997) Curr. Opin. Str. Biol. 7:117-125). The amino acid sequences (SEQ ID NOS.: 8, 62-64, 7 & 5) of the variant α-helical regions from the selected zinc fingers are: REDVLIRHGK (x) (SEQ ID NO.: 5), RADALMVHKR (y) (SEQ ID NO.: 6), and RGPDLARHGR (z) (SEQ ID

NO.: 7). The DNA sequences (SEQ ID NOS.: 55, 44 & 56) contain the generic binding site GCGGNGGCG (SEQ ID NO.: 4), where the central (bold) nucleotide was either: uracil (U), thymine (T), cytosine (C), or 5-methylcytosine (M).

Page 14, paragraph beginning at line 16 is amended as follows:

Figure 2 shows the effect of cytosine methylation on DNA binding by phage-selected zinc fingers. Graphs show three different zinc finger phage binding to the DNA sequence (SEQ ID NO.: 43) GCGGCGGCG in the presence (circle) and absence (triangle) of methylation of the central base (bold). The zinc finger clones tested contained variant α-helical regions of the middle finger as follows: (a) RADALMVHKR (SEQ ID NO.: 6), (b) RGPDLARHGR (SEQ ID NO.: 7),and (c) REDVLIRHGK (SEQ ID NO.: 5). These respective zinc finger clones preferentially bind their cognate DNA site in the presence, absence, or regardless of cytosine methylation.

Page 14, paragraph beginning at line 24 is amended as follows:

Figure 3 shows the binding site interactions of 5 zinc finger polypeptides (SEQ ID NOS.: 65-66, 68-69, 71-72, 74-75, 77-78, respectively in order of appearance); selected taking into account cross-strand specificity by overlapping finger [randomisation] randomization, with each of the oligonucleotides used in the selection process. Cross-strand contacts are shown.

Page 30, paragraph beginning at line 28 is amended as follows:

The phage display library is screened with the synthetic binding site GCGGMGGCG, containing a 5-meC base analogue (M). After 5 rounds of selection, zinc finger phage are tested for binding to 5-meC and cytosine in the context of the above site, and those capable of specifically binding the methylated site are sequenced in the region of the zinc finger gene. Two different clones are isolated, which are identical to the DNA-binding domains previously selected using the binding site (SEQ ID NO.: 44) GCGGTGGCG.

Page 31, paragraph beginning at line 4 is amended as follows:

Hence the various zinc finger phage selections described above yield different fingers able to bind the generic DNA sequence (SEQ ID NO.: 4) GCGGNGGCG, where N is either thymine, cytosine or 5-meC. A full complement of fingers is selected for recognition of the cytosine/5-meC pair in the above context, some of which [recognise] recognize one type of base exclusively, while others

bound both bases equally well (Figures 1c and 2).

Pag 34, paragraph beginning at line 17 is amended as follows:

The three-finger DNA-binding domain of transcription factor Zif268 binds the DNA sequence (SEQ ID NO.: 45) GCGTGGGCG. Phage display libraries of this zinc finger domain have been used to elucidate aspects of the base-recognition mechanism of zinc fingers and to select fingers which bind to predetermined DNA sequences. We have constructed a set of phage display libraries in which amino acid positions from both finger 2 (F2) and finger 3 (F3) of Zif268 are simultaneously [randomised] randomized in order to evaluate the effect of inter-finger synergy on the specificity of DNA binding. These libraries, hereafter denoted collectively as LF2/3, contain variants which specifically [recognised] recognized DNA sequences of the form XXXXCGGCG or GXXXCGGCG, where X is any nucleotide.

Page 34, paragraph beginning at line 27 is amended as follows:

The HaeIII and HhaI methyltransferases modify the internal cytosine (shown in bold lettering) of their respective DNA recognition sequences GGCC and GCGC. We therefore designed two DNA oligos, one containing the sequence (SEQ ID NO.: 13) GGCCCGGCG and the other (SEQ ID NO.: 14) GCGCCGGCG, which included the sites required for modification by the respective methylases M.HaeIII or M.HhaI (underlined). The oligos also place these sequences in the context of binding sites that could be used to screen LF2/3 for zinc fingers that specifically [recognise] recognize the modified DNA.

Page 37, paragraph beginning at line 12 is amended as follows:

The four zinc finger clones isolated by phage display using synthetic 5-meC–containing DNA target sites are next tested for binding to enzymatically methylated DNA. In this assay a single DNA fragment is used that incorporates both the (SEQ ID NO.: 13) GGCCCGGCG and the (SEQ ID NO. 14) GCGCCGGCG zinc finger binding site sequences (Figure 6a), which additionally are substrates for methylation by M.HaeIII and M.HhaI respectively. Each zinc finger clone is tested for binding to the DNA before and after DNA modification using one or both methylases.
Figure 6b shows that, in contrast to zfHAE(Y) and zfHHA(Y) which both [recognise] recognize the DNA regardless of the methylation status (as would be expected), zfHAE(M) and zfHHA(M) bind only after specific methylation of the DNA by the appropriate methylase enzyme. Thus enzymatic modification of cytosine to 5-meC

can act as a switch that induces specific protein-DNA complex formation.

Page 38, add the following paragraph beginning at lin 15:

In Table 2, clone zfHAE(M) sequences are represented by SEQ ID NOS.: 35-36, respectively, in order of appearance; clone zfHHA(M) sequences are represented by SEQ ID NOS.: 37-38, respectively, in order of appearance; clone zfHAE(Y) sequences are represented by SEQ ID NOS.: 46-47, respectively, in order of appearance; and clone zfHHA(Y) sequences are represented by SEQ ID NOS.: 48-49, respectively, in order of appearance.

Page 39, paragraph beginning at line 8 is amended as follows:

This is the case for the DNA binding site (SEQ ID NO.: 50) GGMCCGGCG in which the 5-meC base (bold) is discriminated from thymine by zinc finger clone zfHAE(M). According to the conventional model of zinc finger-DNA recognition, based on the crystal structure of the Zif268–DNA complex and subsequent biochemical experiments, the 5-meC base in the binding site is contacted by the glutamine residue in α-helical position +6 of finger 2 (Figure 3). Additionally, the complementary guanine can be [recognised] recognized using a synergistic contact from the histidine residue in α-helical position +2 of finger 3 (Figure 3).

Page 40, add the following paragraph beginning at line 14:

Clone zfHAE(M) sequences are represented by SEQ ID NOS.: 35 and 51, respectively, in order of appearance; clone zfHHA(M) sequences are represented by SEQ ID NOS.: 37 and 52, respectively, in order of appearance; and clone zfHAE(Y) sequences are represented by SEQ ID NOS.: 53-54, respectively, in order of appearance.

Page 40, paragraph beginning at line 24 is amended as follows:

A method of converting zinc finger DNA-binding domains to chimaeric restriction endonucleases has been described in Kim, *et al.*, (1996) Proc. Natl. Acad. Sci. USA 93:1156-1160. In order to demonstrate the applicability of methylcytosine-specific zinc fingers to restriction enzymes, a fusion is made between the catalytic domain of Fok I as described by Kim *et al.* and the 5-meC specific zinc finger described in Example 3. Fusions of the 5-meC zinc finger nucleic acid-binding domain to the catalytic domain of Fok I restriction enzyme results in a novel endonuclease which cleaves DNA adjacent to the DNA recognition sequence of the zinc finger, namely (SEQ ID NO.: 55) GCGGMGGCG.

Page 41, paragraph b ginning at line 1 is amend d as follows:

The oligonucleotides (SEQ ID NO.: 55) GCGGMGGCG and (SEQ ID NO.: 43) GCGGCGGCG are [synthesised] synthesized and ligated to random DNA sequences. After incubation with the zinc finger restriction enzyme, the nucleic acids are [analysed] analyzed by gel electrophoresis. Bands indicating cleavage of the nucleic acid at a position corresponding to the location of the oligonucleotide (SEQ ID NO.: 55) GCGGMGGCG are visible with the methylated, but not the unmethylated, nucleic acid.

Page 41, paragraph beginning at line 7 is amended as follows:

In a further experiment, the 5-meC-specific zinc finger is fused to an amino terminal copper/nickel binding motif. Under the correct redox conditions (Nagaoka, M., *et al.*, (1994) J. Am. Chem. Soc. 116:4085-4086), sequence-specific DNA cleavage is observed, only in the presence of 5-meC containing DNA incorporating the oligonucleotide (SEQ ID NO.: 55) GCGGMGGCG.

Page 41, paragraph beginning at line 25 is amended as follows:

Thus, a zinc finger which [recognises] recognizes the DNA sequence (SEQ ID NO.: 56) GCGGCCGCG selected by phage display as described in Choo, Y. & Klug, A. (1994) Proc. Natl. Acad. Sci. U.S.A. 91:11163-11167. By the method of the preceding examples, a further zinc finger is selected which is capable of binding to the sequence (SEQ ID NO.: 54) GCGGMCGCG where the central base M is 5-meC, and used to construct transcription factors as described in the foregoing.

Page 42, paragraph beginning at line 1 is amended as follows:

A transient expression experiment is conducted, wherein the CAT reporter gene on the reporter plasmid is placed downstream of the sequence (SEQ ID NO.: 56) GCGGCCGCG. The reporter plasmid is cotransfected with a plasmid vector expressing the zinc finger-HSV fusion under the control of a constitutive promoter. No activation of CAT gene expression is observed.

Page 42, paragraph beginning at line 7 is amended as follows:

However, when the same experiment is conducted in the presence of Hae III methylase, CAT expression is observed as a result of the methylation of (SEQ ID NO.: 56) GCGGCCGCG to form (SEQ ID NO.: 58) GCGGMCGCG, and consequent binding of the zinc finger transcription factor to its recognition sequence.

IN THE CLAIMS:

Claim 15 has been amended as indicated below.

15. (Twice Amended) The method according to claim 10, wherein the linker is the sequence set forth in SEQ ID NO.: 41 [T-G-E-K] or the sequence set forth in SEQ ID NO.:3.